Amendments to the Specification

Please amend the paragraph appearing on page 7, line 21 to page 8, line 7 as follows:

--Within one embodiment, the signal peptide sequence is the native sequence of the protein to be trimerized. Within another embodiment, the signal peptide sequence ~~is~~ originates from a secreted protein different from that to be trimerized. Within one embodiment, the non-collagen polypeptide to be trimerized is a soluble receptor consisting of the ligand binding domain(s). Within one embodiment, the C-terminal portion of collagen is the C-propeptide without any triple helical region of collagen (~~SEQ ID NOS:3-4~~ SEQ ID NOs:3-4). Within another embodiment, the C-terminal collagen consists of a portion of the triple helical region of collagen as linker to the non-collagenous proteins to be trimerized (~~SEQ ID NOS:1-2~~ SEQ ID NOs:1-2). Within another embodiment, the C-terminal portion of collagen has a mutated or deleted BMP-1 protease recognition site (~~SEQ ID NOS: 3-4~~ SEQ ID NOs:3-4).--

Please amend the paragraph appearing on page 8, lines 19-23 as follows:

--In a preferred embodiment, the non-collagen polypeptide to be trimerized is the soluble TNF-RII (p75) (~~SEQ ID NOS:9-12~~ SEQ ID NOs:9-12). In another preferred embodiment, the non-collagen polypeptide to

be trimerized is soluble CD-4, the co-receptor of HIV (~~SEQ ID NOS:13-16~~ SEQ ID NOs:13-16). In yet another preferred embodiment, the non-collagen polypeptide to be trimerized is a placental secreted alkaline phosphatase (~~SEQ ID NOS:5-8~~ SEQ ID NOs:5-8).--

Please amend the paragraph appearing on page 22, lines 4-15 as follows:

--To demonstrate the feasibility for making secreted trimeric fusion proteins, cDNA sequences encoding the entire C-propeptides of human α1(I) containing either some glycine-repeat triple helical region (T0 construct, ~~SEQ ID NOS:1-2~~ SEQ ID NOs:1-2), or no glycine-repeat with a mutated BMP-1 recognition site (T2 construct, ~~SEQ ID NOS:3-4~~ SEQ ID NOs:1-2) were amplified by RT-PCR using EST clones purchased from the American Type Culture Collection (ATCC). The amplified cDNAs were each cloned as a Bgl II-XbaI fragment into the pAPtag2 mammalian expression vector (GenHunter Corporation; Leder et al., 1996 and 1998), replacing the AP coding region (Fig. 2). The resulting vectors are called pTRIMER, versions T2 and T0, respectively. The vectors allow convenient in-frame fusion of any cDNA template encoding a soluble receptor or biologically active protein at the unique Hind III and Bgl II sites. Such fusion proteins have the collagen trimerization tags located at the C termini, similar to native pro-collagens. --

Please amend the paragraph appearing on page 22, line 17 to page 23, line 6 as follows:

--To demonstrate the feasibility of this invention, a cDNA encoding the human secreted placental alkaline phosphatase (AP), including its native signal peptide sequence, was cut out as a Hind III-Bgl II fragment from the pAPtag4 vector (GenHunter Corporation; Leder et al., 1996 and 1998) and cloned into the corresponding sites of the pTRIMER-T0 and pTRIMER-T2 vectors. The resulting AP-collagen fusion constructs (~~SEQ ID NOS:5-8~~ SEQ ID NOs:5-8) were expressed in HEK293T cells (GenHunter Corporation) after transfection. The successful secretion of the AP-collagen fusion proteins can be readily determined by AP activity assay using the conditioned media of the transfected cells. The AP activity reached about 1 unit/mL (or equivalent to about 1 µg/mL of the fusion protein) 2 days following the transfection. To obtain HEK293T cells stably expressing the fusion proteins, stable clones were selected following co-transfection with a puromycine-resistant vector, pBabe-Puro (GenHunter Corporation). Clones expressing AP activity were expanded and saved for long-term production of the fusion proteins.--

Please amend the paragraph appearing on page 23, line 20 to page 24, line 15 as follows:

--To provide a proof that new and therapeutically beneficial biological functions can be endowed to a trimeric fusion protein, next a trimeric human soluble TNF-RII (p75) receptor using a corresponding EST clone purchased from the ATCC was constructed. As described in Example 1, the N-terminal region of human TNF-RII, including the entire ligand-binding region, but excluding the trans-membrane domain, was cloned in-frame, as a Bam H I fragment, into the Bgl II site of both pTRIMER-T0 and pTRIMER-T2 vectors (~~SEQ ID NOS:9-12~~ SEQ ID NOs:9-12). The resulting fusion constructs were expressed in HEK293T cells following transfection. Stable clones were obtained by puromycine co-selection as described in Example 1. Western blot analysis under both non-reducing and reducing conditions was carried out to determine if the resulting soluble TNF-RII-collagen fusion proteins were indeed expressed, secreted and assembled into trimeric forms. As expected, the monoclonal antibody against human TNF-RII (clone 226 from R & D Systems, Inc.) clearly recognized the trimeric soluble TNF-fusion proteins expressed by both T0 and T2 fusion

vectors as 220-240 kDa bands, which are about three times bigger than the corresponding monomeric fusion proteins (Fig. 3B). The TNF-RII antibody failed to detect monomeric fusion proteins under reducing conditions, consistent with the property specified by the antibody manufacturer. As a negative control for antibody specificity, neither the HEK293T cell alone, nor the cells expressing AP-T2 fusion protein expressed any TNF-RII (Fig. 3B).—

Please amend the paragraph appearing on page 26, line 18 to page 27, line 5 as follows:

--To create such trimeric soluble CD4 HIV receptor analogs, a cDNA encoding the entire human soluble CD4, including its native signal peptide sequence, but excluding the transmembrane and the short cytoplasmic domains, was amplified using an EST clone purchased from the ATCC. The resulting cDNA was then cloned as a Hind III-Bgl II fragment into the corresponding sites of the pTRIMER-T0 and pTRIMER-T2 expression vectors. The resulting soluble CD4-collagen fusion constructs (~~SEQ ID NOS:13-16~~ SEQ ID NOs:13-16) were expressed in HEK293T cells (GenHunter Corporation) after transfection. To obtain HEK293T cells stably

expressing the fusion proteins, stable clones were selected following co-transfection with a puromycine-resistant vector, pBabe-Puro (GenHunter Corporation). Clones expressing the fusion proteins were expanded and saved for long-term production of the fusion proteins.--